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PROCEDURES FOR SCREENING AND TESTING ALFALFA FOR RESISTANCE TO THE ALFALFA SEED CHALCID

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Thousands of alfalfa plants have been screened for resistance to the alfalfa seed chalcid (Bruchophagus roddi Gussakovskii) at Mesa, Ariz., since 1958. This research has resulted in the development of procedures for identifying chalcid-resistant plants and determining their levels of resistance. This report describes these methods and suggests precautions for those planning a screening program.

In general, the procedures for screening and testing for insect resistance consist of three phases. The initial phase requires the exposure of plant material of diverse origin either to natural insect populations in the field or to artificial caged populations. In this study, exposure in the field was employed to screen as large a number of plants as possible with a minimum of effort and funds. The second phase is forced association of the test insect with the plants selected as resistant in phase one. Small cages similar to those developed by Strong[1/] were used to confine chalcids on the racemes of tripped flowers of the proper age. The third phase is a progeny test, which permitted final acceptance or rejection of the parent plant material on the basis of its capacity to produce resistant progeny.

Field screening of plants was started by planting seed in rows 3 feet apart and thinning the seedlings within rows 18 to 24 inches apart. The larger seedlings were retained, smaller plants were hoed out. This selective thinning permitted early selection of the more vigorous material and decreased the need for subsequent agronomic evaluations. As the plants matured, they were allowed to set seed so that when chalcid populations were maximum, the condition of the pods was optimum for oviposition. In Arizona, maximum chalcid infestations occur in August; thus to coordinate pod development and chalcid population, the last cutting was made 6 to 8 weeks earlier.

1/ Strong, F. E. Laboratory studies of the biology of the alfalfa seed chalcid Bruchophagus roddi Guss. (Hymenoptera: Eurytomidae). Hilgardia 32(3):229-249. 1962.

The sample taken from each plant was arbitrarily considered to represent 25 racemes. Only racemes having green, fully expanded pods were selected to lessen variability of data caused by pod development. This uniformity of samples was stressed during the mechanical process of collecting the racames, because the age of the pods was found to contribute most of the variation.

Each sample was placed in a special rearing container (fig. 1), a pint-sized ice cream carton fitted with a single 1-dram vial inserted into a rubber gasket and glued to the center of the lid. The containers were placed in parallel position on a portable rack that held 200 cartons (fig. 2). Thus a series of racks could be used to process as many as 3,000 samples simultaneously. Cartons were left on the racks for 3 weeks or until adult emergence ceased. Then the vials were removed, the chalcids within were counted, and the number was recorded. Each carton was also examined, and the chalcids were counted.

Figure 1.-- Pint-sized ice cream carton used to rear adults of alfalfa seed chalcid.

Figure 2.--Rack holding about 200 rearing cartons.

Samples taken during August at Mesa produced about 90 percent chalcid emergence in 3 to 4 weeks; 10 percent entered diapause and emerged the following March or April. Conversely, samples taken in October or November at Mesa produced about 10 percent emergence in 3 to 4 weeks, and 90 percent entered diapause and emerged the next spring. Samples should therefore be taken early if results of the test are desired in a few weeks.

Selection of promising plants was based on the number of adult chalcids emerging from the sample. If less than 25 adults emerged from a sample, the plant represented by the sample was retained for cage testing in the greenhouse. On this basis, about 10 percent of the original plant population was selected for further testing.

Rooted cuttings of each plant selected as resistant in phase one were established in perlite and transplanted individually to 5-inch clay pots. The potted plants were maintained on benches in the greenhouse at 80° to 90° F. They were cut back several times before flowering to stimulate crown growth and increase stem development. When they were allowed to bloom, the flowers were tripped at random by hand to insure cross pollination (fig. 3). A schedule of 16 hours of light plus artificial luminescence was used to decrease vegetative growth and increase flower production. Pod development was a critical factor. Raceme retardation caused premature drying and hardening of the pods, but the condition could sometimes be prevented by proper watering and fertilization of the plants with ammonium phosphate every 2 weeks.

Figure 3.--Pollinating alfalfa flowers.

The chalcids for the cage testing in the greenhouse were reared from infested seed obtained from cold storage at 40° F. before use and stored in a special rearing chamber, 15 by 30 by 36 inches, constructed of wood and glass (fig. 4). The upper half was sealed with glass, and the lower section held wooden trays containing the seed. Flourescent tubes were attached to the outside of the chamber near the top. As the chalcids emerged, they accumulated on the inside of the glass near the lights. A mouth aspirator could be inserted through either of the two sleeved openings in front of the chamber to collect the chalcids (fig. 5). After the chalcids were aspirated from the chamber, they were placed in half-gallon ice cream cartons coated with honey and stored temporarily in a refrigerator at 50° F. Adult survival was 3 to 4 weeks with and 2 to 3 days without honey provided as food. Full trays held about 10 pounds of seed, and 3,000 to 4,000 adults were produced within 2 to 3 weeks after the seed was placed in the rearing chamber.

Figure 4.--Chamber for mass-rearing alfalfa seed chalcids from infested seed screenings.

Figure 5.--Removing chalcids from rearing chamber.

The cages used to confine the chalcids to racemes were made by halving 5-inch-long cellulose nitrate centrifuge tubes (fig. 6). Each cage was secured to a raceme by a disk of plastic foam provided with a radial slit to attach to the rachis of the racemes (fig. 7). Another disk was inserted in the opposite end to close the cage (fig. 8). The chalcids were introduced through a small opening drilled on one side of the tube section. A small cork tipped with honey plugged the side opening. The honey was periodically renewed by withdrawing the cork. Five to ten of the racemes on each plant were selected for cage testing when the pods were from 10 to 15 days old. Ten adult chalcids were introduced into each cage (fig. 9) and left in the cages for 2 weeks. Feeding was accomplished by periodically renewing the honey.

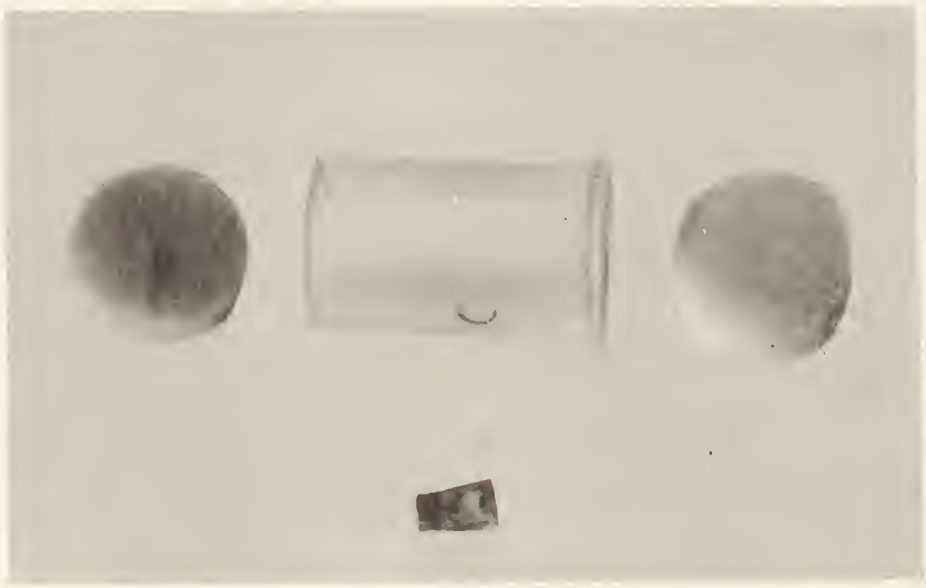

Figure 6.--Unassembled cage used for confining chalcids to alfalfa racemes

Figure 7.--Foam disk attached to rachis of raceme.

Figure 8.--Completed cage attached to alfalfa raceme.

Figure 9.--Introducing chalcids into cage.

After the test period, the cages were removed from the racemes, and the racemes were clipped off the plants and placed in rearing cartons. The plants were cut back, fertilized, and watered in preparation for the next test. Two weeks after the racemes had been placed in the rearing cartons, the chalcids that emerged were counted and recorded. Plants that averaged one or more chalcids per raceme were discarded; those with less than one chalcid per raceme were given a second test.

Cage tests were repeated three times on each plant that showed promise of resistance. For each group of test plants, susceptible checks were included to compare the level of infestations (fig. 10). Final selection of plants was based on the infestation level of an average of one adult chalcid or less per raceme for each of the three cage tests (fig. 11), a level of infestation of about 5 percent. Plants finally selected for resistance represented about 1 percent of the number exposed during the original field tests.

Figure 10.--Group of test plants with susceptible checks.

Figure 11.--Population of chalcids reared from resistant plant (left) and susceptible check (right). Five chalcids emerged from resistant plant and 66 from susceptible plant.

Progeny tests were conducted in the field with seed produced from a restricted polycross among parent plants. The method was similar to that used in the phase one field screening. However, in testing progeny for chalcid resistance it is necessary to isolate the test progeny from the check plants to avoid intercrossing and subsequent contamination of material from the susceptible check plants. Otherwise, it is difficult to get an accurate appraisal of the level of resistance in the progeny and to determine whether chalcid resistance is passed from parent to progeny.

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